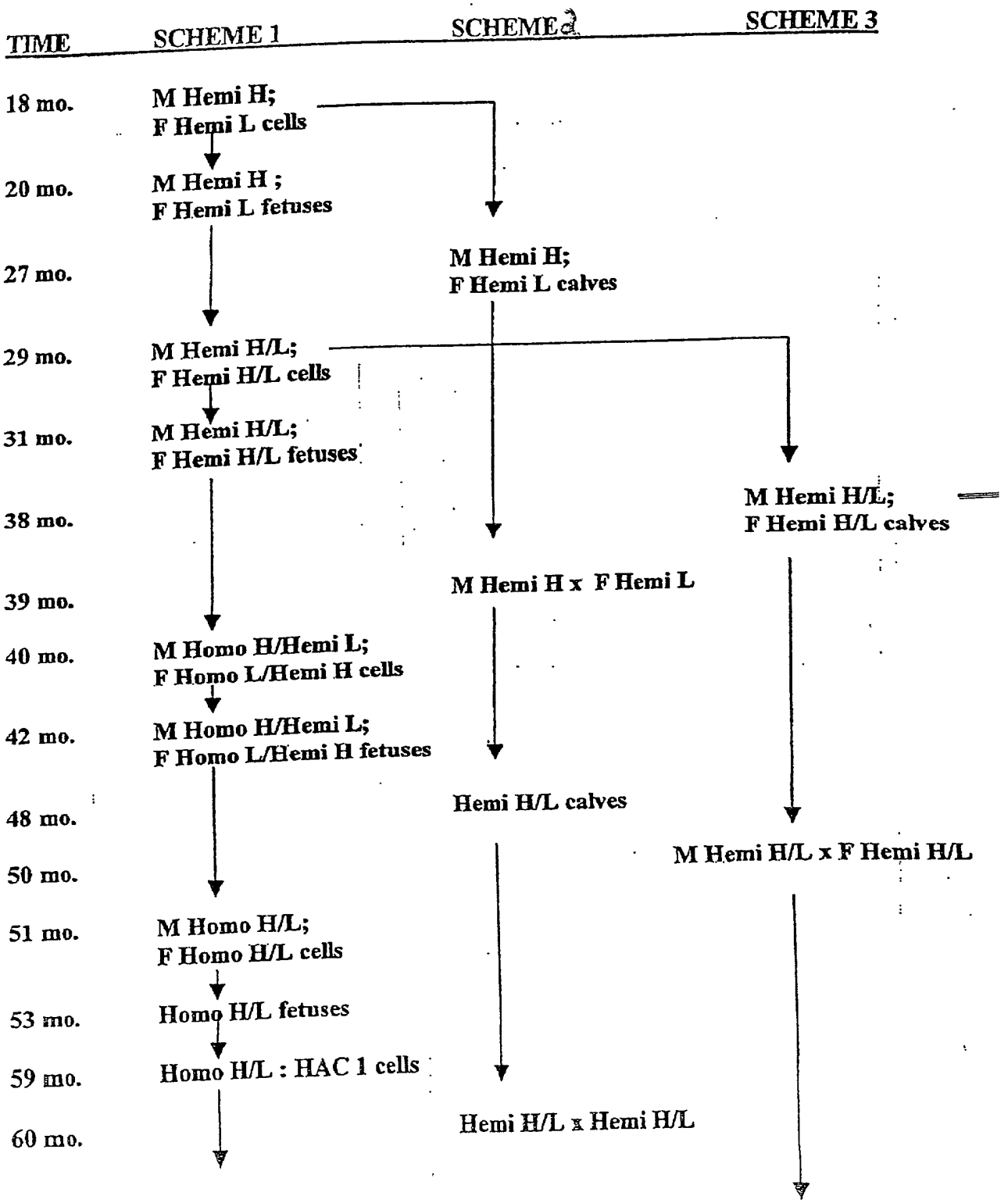

TIME
SCHEME 1
SCHEME 2
SCHEME 3
18 mo.
M Hemi H;
F Hemi L cells
20 mo.
M Hemi H ;
F Hemi L fetuses
27 mo.
M Hemi H;
F Hemi L calves
29 mo.
M Hemi H/L;
F Hemi H/L cells
31 mo.
M Hemi H/L;
F Hemi H/L fetuses
38 mo.
M Hemi H/L;
F Hemi H/L calves
39 mo.
M Hemi H x F Hemi L
40 mo.
M Homo H/Hemi L;
F Homo L/Hemi H cells
42 mo.
M Homo H/Hemi L;
F Homo L/Hemi H fetuses
48 mo.
Hemi H/L calves
50 mo.
M Hemi H/L x F Hemi H/L
51 mo.
M Homo H/L;
F Homo H/L cells
53 mo.
Homo H/L fetuses
59 mo.
Homo H/L : HAC 1 cells
60 mo.
Hemi H/L x Hemi H/L

Figure 1A

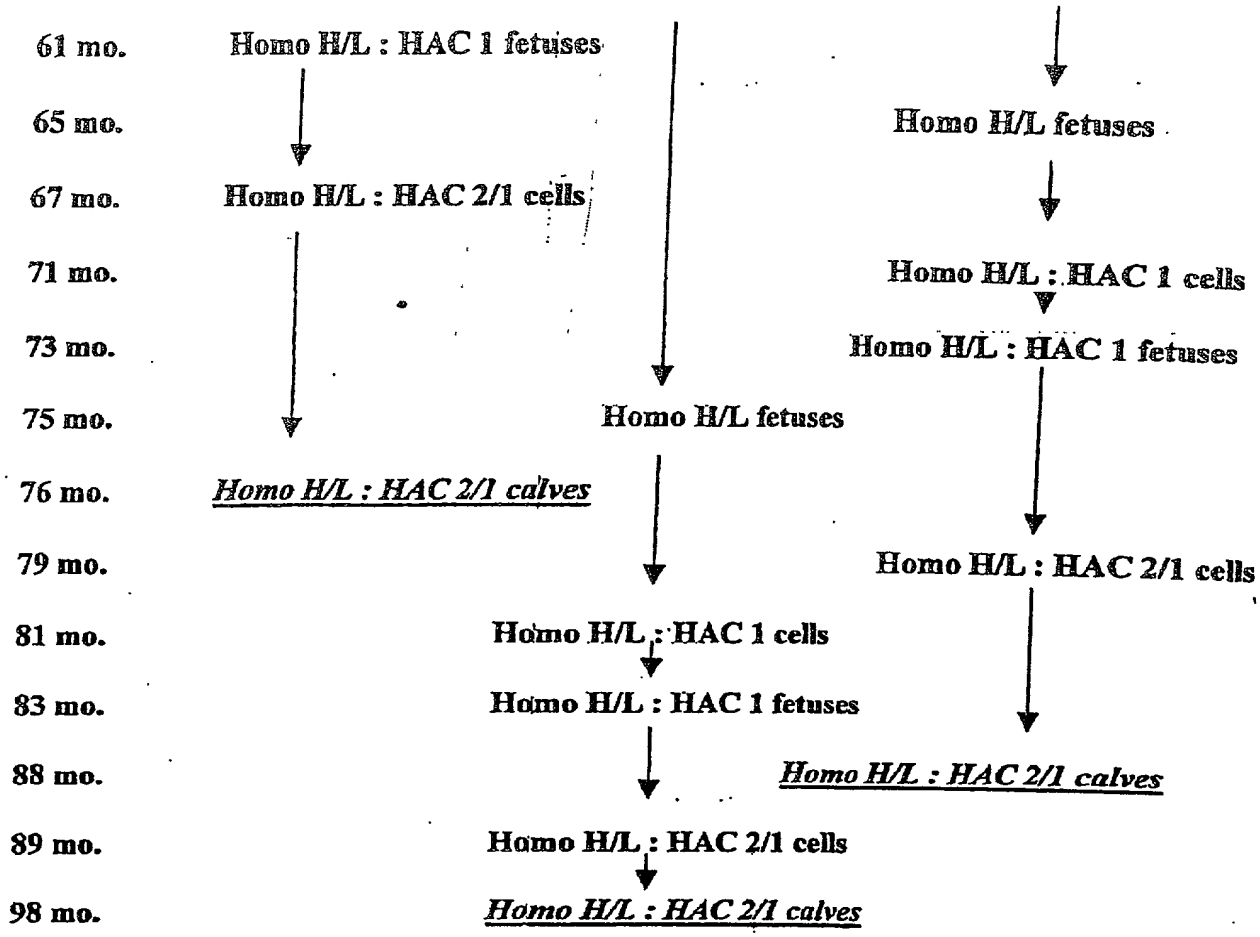


Figure 1A continued

TIME	SCHEME 1
27 mo.	M Hemi H; F Hemi H cells
29 mo.	M Hemi H ; F Hemi H fetuses
32 mo.	M Homo H; F Homo H cells
34 mo.	M Homo H; F Homo H fetuses
37 mo.	M Homo H/Hemi L; F Homo H/Hemi L cells
39 mo.	M Homo H/Hemi L; F Homo H/Hemi L fetuses
42 mo.	M Homo H/L; F Homo H/L cells
44 mo.	Homo H/L fetuses

Figure 1B

47 mo. Homo H/L : Δ or ΔΔHAC cells

↓

49 mo. Homo H/L : Δ or ΔΔHAC fet

↓

58 mo. Homo H/L; Δ or ΔΔHAC calf

Figure 1B continued

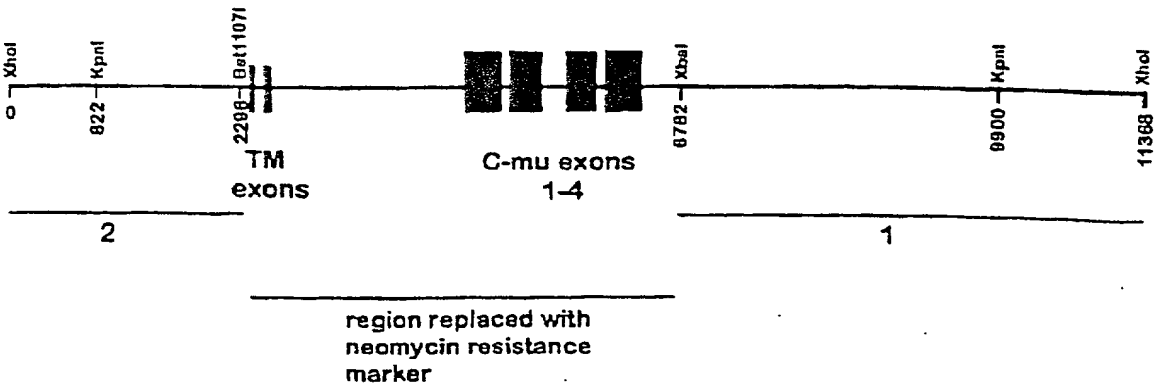

XhoI
KpnI
Bst1107I
0
822
2298
TM
exons
C-mu exons
1-4
6782
XbaI
9900
KpnI
11368
XhoI
2
1
region replaced with
neomycin resistance
marker

Figure 2A

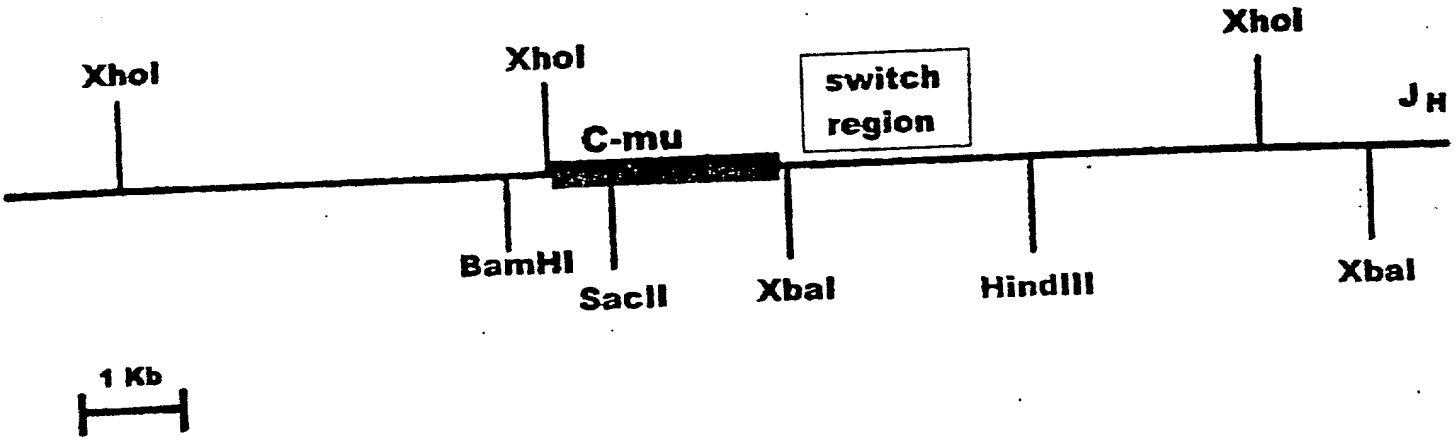
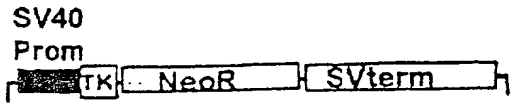
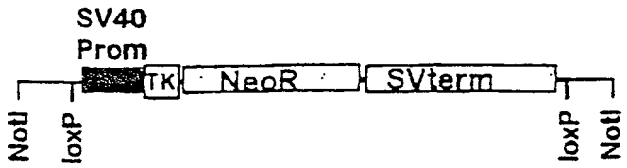

XhoI
XhoI
C-mu
switch
region
XhoI
J H
BamHI
SacII
XbaI
HindIII
XbaI
1 Kb

Figure 2B

A



B



Figures 3A and 3B

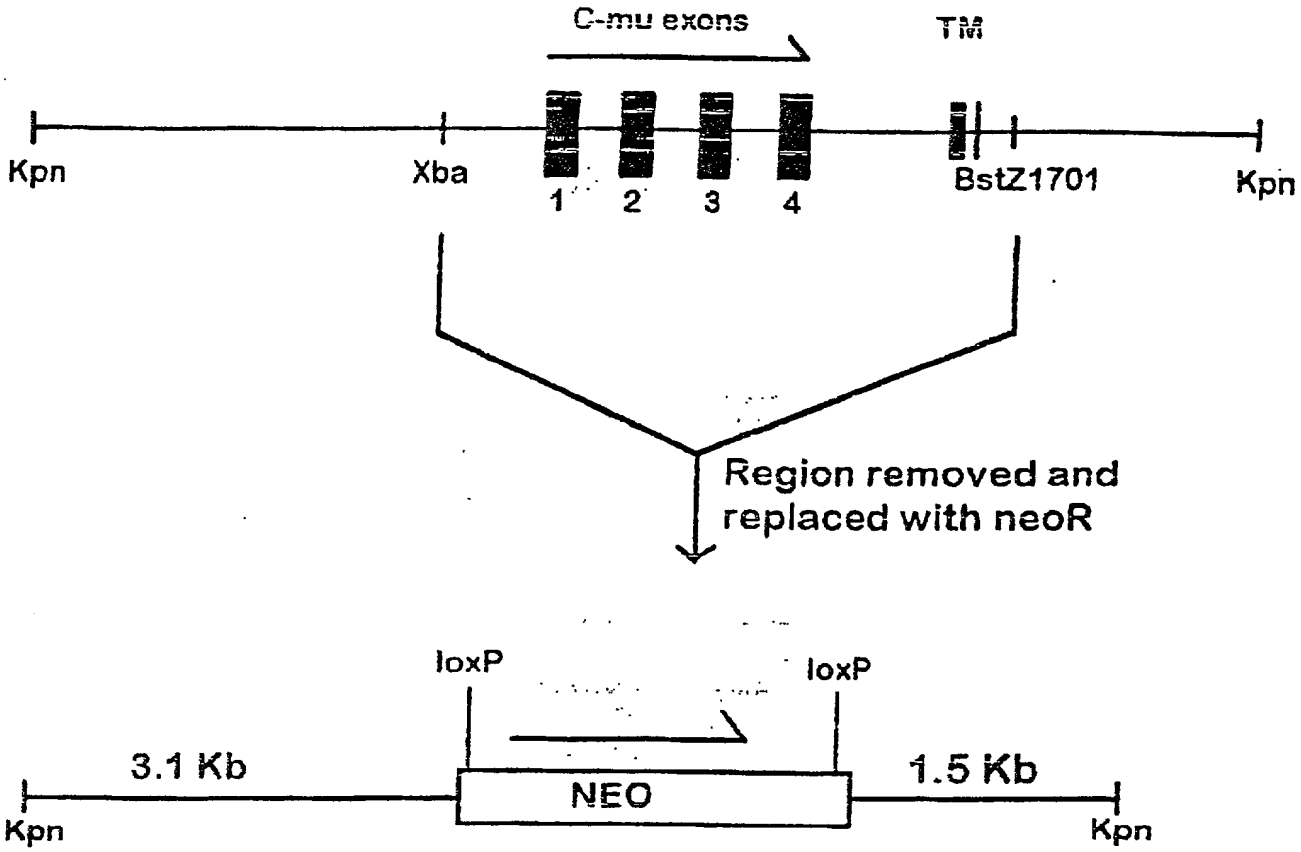

C-mu exons
TM
Kpn
Xba
1
2
3
4
BstZ1701
Kpn
Region removed and
replaced with neoR
loxP
loxP
3.1 Kb
NEO
1.5 Kb
Kpn
Kpn

Figure 3C

SEQ ID NO: 47

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Figure 3D

SEQ ID NO:48

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ccagccctgctcaactcagcccagcacagcccaacttggctcagctcagcttagcccagctcagcccagcttacccactccgcc
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nnn

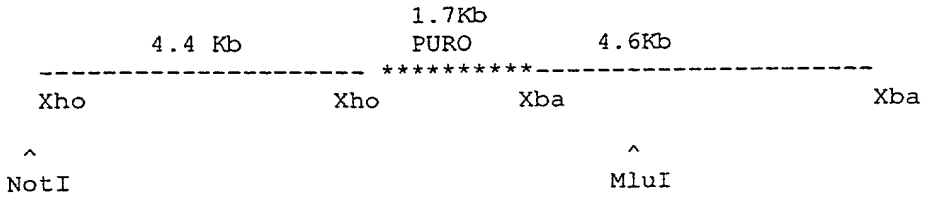


Figure 3F

SEQ ID NO:60

```
   1  atgagattcc ctgctcagct cctggggctc ctcctgctct gggtcccagg
  51  atccagtggg gatgttgtgc tgacccagac tcccctctcc ctgtctatca
 101  tccctggaga gacggtctcc atctcctgca agtctactca gagtctgaaa
 151  tatagtgatg gaaaaaccta tttgtactgg cttcaacata aaccaggcca
 201  atcaccacag cttttgatct atgctgtttc cagccgttac actggggtcc
 251  cagacaggtt cactggcagt gggtcagaaa cagatttcac acttacgatc
 301  aacagtgtgc aggctgagga tgttggagtc tattactgtc ttcaaacaac
 351  atatgtccca aatactttcg gccaaggaac caaggtagag atcaaaaggt
 401  ctgatgctga gccatccgtc ttcctcttca aaccatctga tgagcagctg
 451  aagaccggaa ctgtctctgt cgtgtgcttg gtgaatgatt tctaccccaa
 501  agatatcaat gtcaagtgga aagtggatgg ggttactcag agcagcagca
 551  acttccaaaa cagtttcaca gaccaggaca gcaagaaaag cacctacagc
 601  ctcagcagca tcctgacact gcccagctca gagtaccaaa gccatgacgc
 651  ctatacgtgt gaggtcagcc acaagagcct gactaccacc ctcgtcaaga
 701  gcttcagtaa gaacgagtgt tag
```

Figure 3G

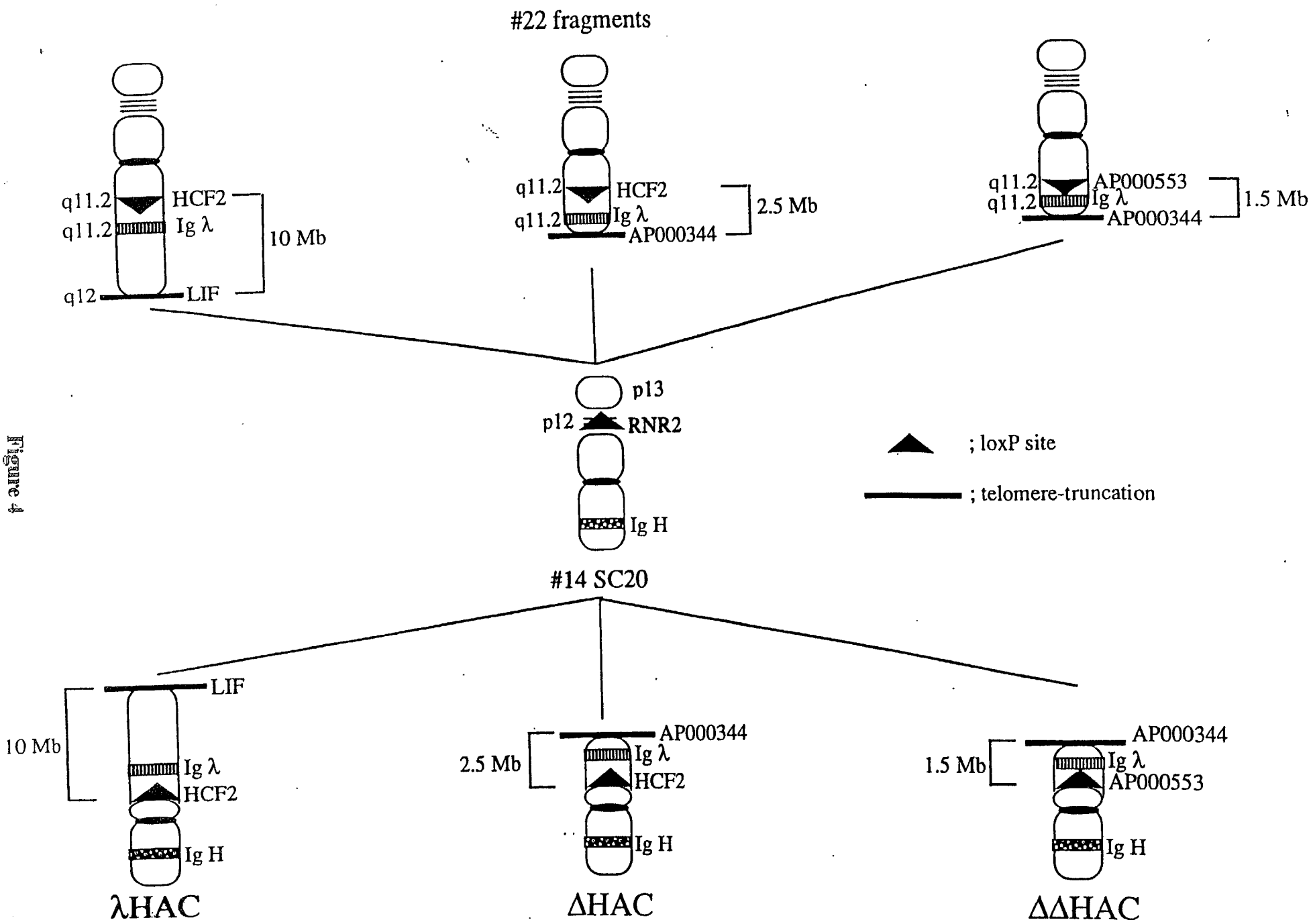
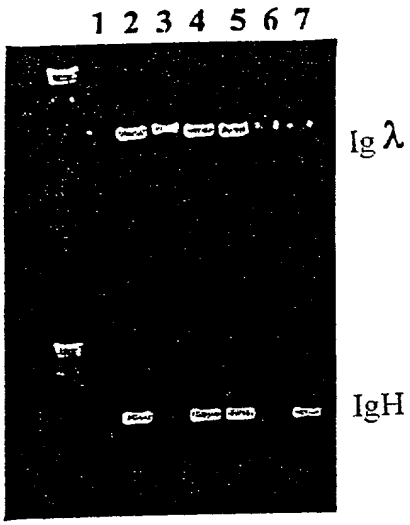


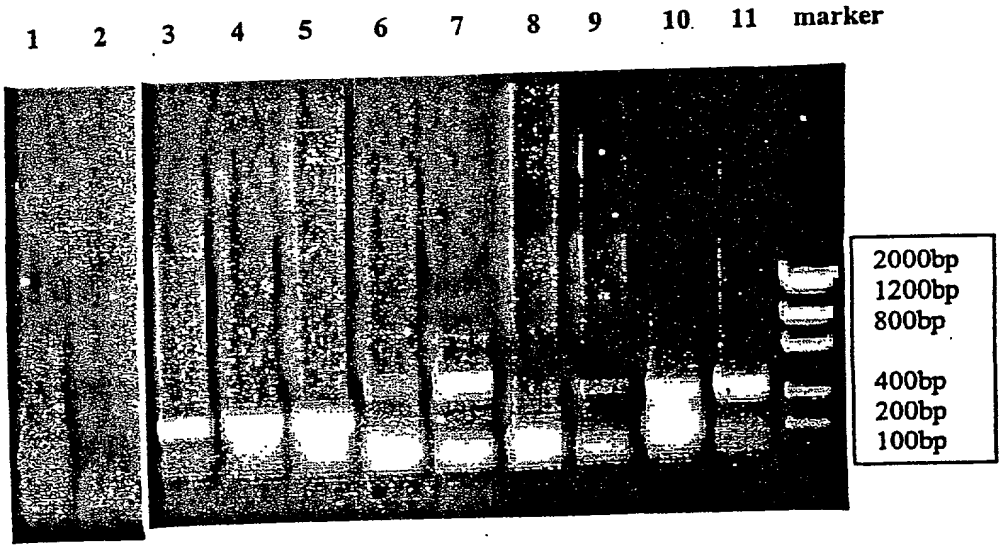
Figure 4



1. Bovine genomic DNA (negative control)
2. Fetus 5968 genomic DNA at 56 days
3. Fetus 5983 genomic DNA at 56 days
4. Fetus 6032 genomic DNA at 58 days
5. Fetus 6045 genomic DNA at 56 days
6. Fetus 5846 genomic DNA at 79 days
7. Fetus 5996 genomic DNA at 77 days

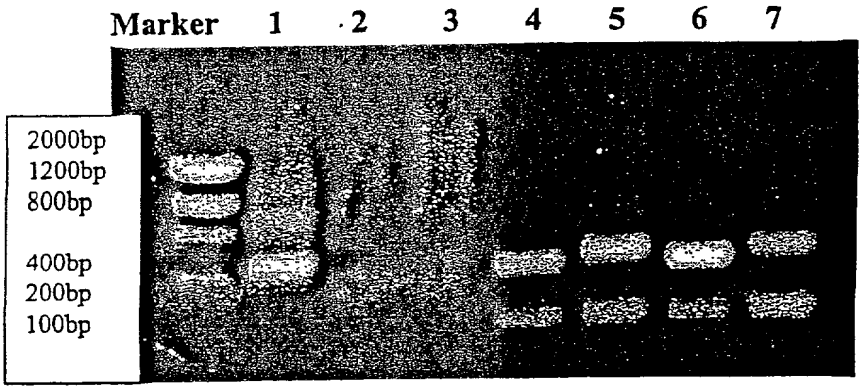
Fetus	Clone	IgH	Ig λ
5968	B4-2	Pos	Pos
5983	B2-13	Neg	Neg
6032	B4-8	Pos	Pos
6045	B2-22	Pos	Pos
5846	B4-8	Neg	Neg
5996	B4-2	Pos	Neg

Figure 5



1. Human mu constant region in bovine liver cDNA from fetus 5996.
2. Human mu constant region in bovine brain cDNA from fetus 5996.
3. Human mu constant region in bovine spleen cDNA from fetus 5996.
4. Human mu constant region in human spleen cDNA.
5. Human mu constant region in mouse spleen CDNA with HAC.
6. Bovine rearranged Cmu heavy chain in bovine spleen cDNA from fetus 5996.
7. Bovine rearranged Cmu heavy chain in human spleen cDNA.
8. Bovine rearranged Cmu heavy chain in mouse spleen CDNA with HAC.
9. GAPDH primers in bovine spleen cDNA from fetus 5996.
10. GAPDH primers in bovine liver cDNA
11. GAPDH primers in mouse spleen CDNA with HAC.

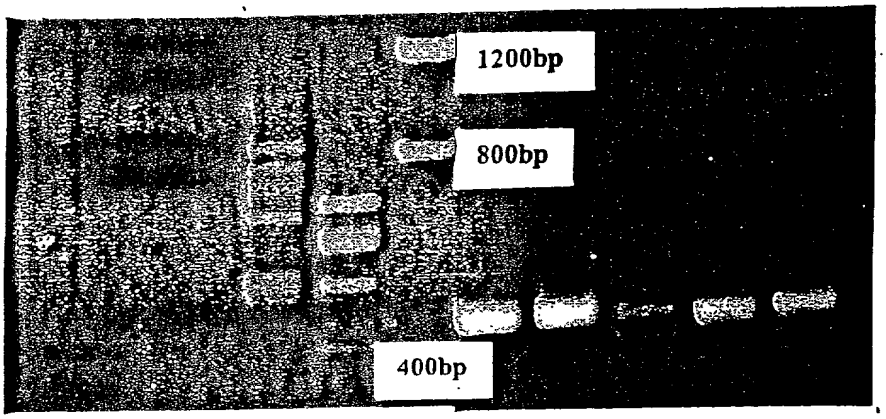
Figure 6



1. GAPDH primers in bovine liver cDNA
2. Bovine rearranged Cmu heavy chain in bovine brain cDNA from fetus 5996.
3. Bovine rearranged Cmu heavy chain in bovine liver cDNA from fetus 5996.
4. GAPDH primers in bovine spleen cDNA from fetus 5996.
5. Bovine rearranged Cmu heavy chain in bovine spleen cDNA from fetus 5996.
6. GAPDH primers in in bovine brain cDNA from fetus 5996.
7. Bovine rearranged Cmu heavy chain positive control.

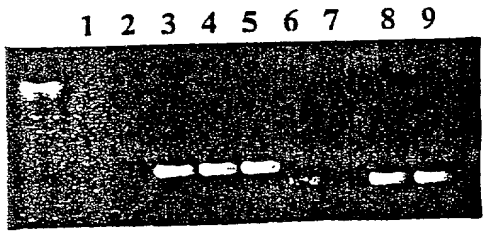
Figure 7

1 2 3 4 5 marker 6 7 8 9 10



1. Human rearranged Cmu heavy chain in mouse spleen CDNA with HAC (+ control).
2. Human rearranged Cmu heavy chain in bovine liver cDNA from fetus.
3. Human rearranged Cmu heavy chain in bovine brain cDNA from fetus 5996
4. Human rearranged Cmu heavy chain in human spleen cDNA (+ control).
5. Human rearranged Cmu heavy chain in bovine spleen cDNA from fetus 5996.
6. GAPDH primers in bovine spleen cDNA from fetus 5996.
7. GAPDH primers in in mouse spleen CDNA with HAC
8. GAPDH primers in bovine brain cDNA from fetus 5996.
9. GAPDH primers in bovine liver cDNA from fetus 5996.
10. GAPDH primers positive control.

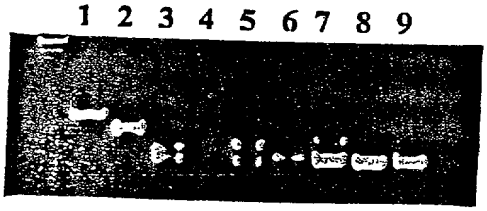
Figure 8



1. Mouse spleen (negative control)
2. Bovine spleen (negative control)
3. Fetus 5996 brain
4. Fetus 5996 liver
5. Fetus 5996 liver
6. Fetus 5996 spleen
7. Fetus 5996 spleen
8. ΔHAC-chimeric mouse spleen (positive control)
9. Human spleen (positive control)

Unspliced genomic fragment
Spliced transcript

Figure 9



1. Mouse spleen (negative control)
2. Bovine spleen (negative control)
3. Fetus 5996 brain
4. Fetus 5996 liver
5. Fetus 5996 liver
6. Fetus 5996 spleen
7. Fetus 5996 spleen
8. ΔHAC-chimeric mouse spleen (positive control)
9. Human spleen (positive control)

Figure 10

Figure 11A SEQ ID NO: 49

5'
GGGAAGGAAGTCCTGTGCGACCANCCAACGGCCACGCTGCTCGTATCCGACG
GGGAATTCTCACAGGAGACGAGGGGGAAAAGGGTTGGGGCGGATGCACTCC
CTGAGGAGACGGTGACCAGGGTTCCNTGGCCCCAGNNGTCAAA3'

Figure 11B SEQ ID NOs: 50 and 51

V-D-J region |→**constant mu region**

```
Subject: 5'
tttgactactggggccagggaaccctggtcaccgtctcctcagggagtgcatccgcccca
------nn---------n----------------------------------------
Query

Subject:
acccttttccccctcgtctcctgtgagaattccccgtcggatacgagcagcgtggccgtt
------------------------------------------------------------
Query

Subject: 5'
ggctgcctcgcacaggacttccttcccgactccatcactttctcctg 3'
--n---g------------------- Cmu1 primer
^
```

SEQ ID NOs 52 and 53

```
                10        19        28        37        46        55
5' GGA GGC TTG GTC AAG CCT GGA GGG TCC CTG AGA CTC TCC TGT GCA GCC TCT GGA
   G   G   L   V   K   P   G   G   S   L   R   L   S   C   A   A   S   G

                64        73        82        91       100       109
   TTC ACC TTC AGT GAC TAC TAC ATG AGC TGG ATC CGC CAG GCT CCA GGG AAG GGG
   F   T   F   S   D   Y   Y   M   S   W   I   R   Q   A   P   G   K   G

               118       127       136       145       154       163
   CTG GAG TGG GTT TCA TAC ATT AGT AGT AGT GGT AGT ACC ATA TAC TAC GCA GAC
   L   E   W   V   S   Y   I   S   S   S   G   S   T   I   Y   Y   A   D
                                  VH3-11
               172       181       190       199       208       217
   TCT GTG AAG GGC CGA TTC ACC ATC TCC AGG GAC AAC GCC AAG AAC TCA CTG TAT
   S   V   K   G   R   F   T   I   S   R   D   N   A   K   N   S   L   Y

               226       235       244       253       262       271
   CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC ACG GCT GTG TAT TAC TGT GCG AGA
   L   Q   M   N   S   L   R   A   E   D   T   A   V   Y   Y   C   A   R

               280       289       298       307       316       325
   ATA ACT GGG GAT GCT TTT GAT ATC TGG GGC CAA GGG ACA ATG GTC ACC GTC TCT
   I   T   G   D   A   F   D   I   W   G   Q   G   T   M   V   T   V   S
     D7-27                                 JH3
               334       343       352       361       370       379
   TCA GGG AGT GCA TCC GCC CCA ACC CTT TTC CCC CTC GTC TCC TGT GAG AAT TCC
   S   G   S   A   S   A   P   T   L   F   P   L   V   S   C   E   N   S

                                  CH
               388
   CCG TCG GAT ACG AGC 3'
   P   S   D   T   S
```


Figure 12A

SEQ ID NOs: 54 and 55

GTG GAG TCT GGG GGA GGC TTG GTA CAG CCT GGG AGG TCC CTG AGA CTC TCC TGT
V E S G G G L V Q P G R S L R L S C

GCA GCG TCA GGA TTC ACC TTC AGG AAC TTT GGC ATG CAC TGG GTC CGC CAG GCT
A A S G F T F R N F G M H W V R Q A

VH3-33

CCA GGC AAG GGG CTG GAG TGG GTG ACA GTT ATA TGG TAT GAC GGA AGT AAT CAA
P G K G L E W V T V I W Y D G S N Q

TAC TAT ATA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC AGA GAC AAT TCC AAG
Y Y I D S V K G R F T I S R D N S K

AAC ATG TTG TAT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAT ACG GCT GTG TAT
N M L Y L Q M N S L R A E D T A V Y

TAC TGT GCG AGA-GAT CGC AAT GGC CTG AAG TAC TTC GAT CTC TGG GGC CGT GGC
Y C A R D R N G L K Y F D L W G R G

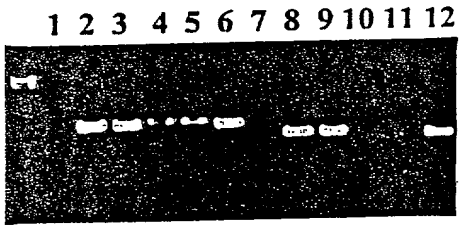
D6-19 ? N addition JH2

ACC CTG GTC ACT GTC TCA TCA GGG AGT GCA TCC GCC CCA ACC CTT TTC CCC CTC
T L V T V S S G S A S A P T L F P L

Cμ

GTC TCC TGT GAG AAT TCC CCG TCG GAT ACG AGC 3'
V S C E N S P S D T S

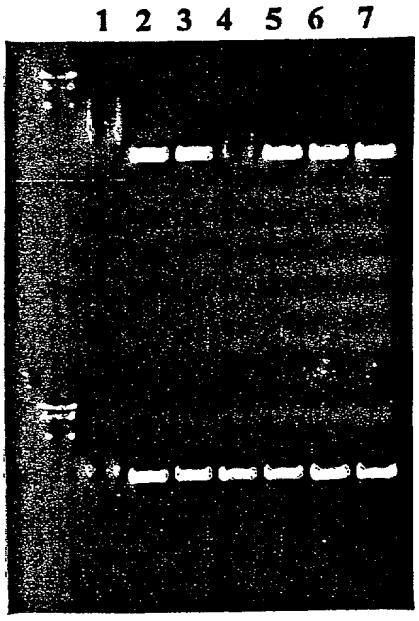
Figure 12B



Fetus	Clone	IgH	Igλ
5580	412	Pos	Pos
5848	214	Neg	Neg

1. Bovine genomic DNA (negative control)
2. Fetus 5580 genomic DNA (Igλ)
3. Fetus 5580 genomic DNA (Igλ)
4. Fetus 5848 genomic DNA (Igλ)
5. Fetus 5848 genomic DNA (Igλ)
6. Positive control (Human genomic DNA)
7. Bovine genomic DNA (negative control)
8. Fetus 5580 genomic DNA (IgH)
9. Fetus 5580 genomic DNA (IgH)
10. Fetus 5848 genomic DNA (IgH)
11. Fetus 5848 genomic DNA (IgH)
12. Positive control (Human genomic DNA)

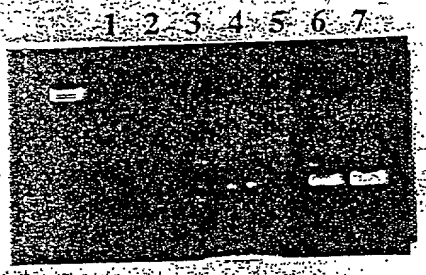
Figure 13



1. Bovine genomic DNA (negative control)
2. Fetus 5442A genomic DNA (91 day)
3. Fetus 5442A genomic DNA (91 day)
4. Fetus 5442B genomic DNA (91 day)
5. Fetus 5442B genomic DNA (91 day)
6. Fetus 5968 genomic DNA (56 day; positive control)
7. Human genomic DNA (positive control)

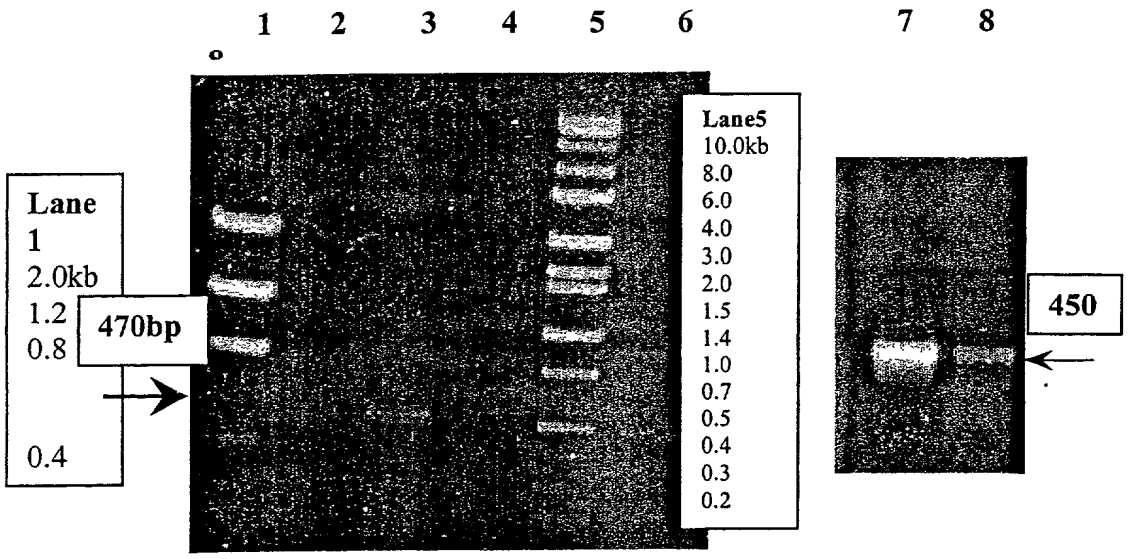
Figure 14

1. Bovine spleen (negative control)
2. Fetus 5442A brain
3. Fetus 5442A liver
4. Fetus 5442A spleen
5. Fetus 5442A spleen
6. Fetus 5996 spleen (positive control)
7. ΔHAC-chimeric mouse spleen (positive control)



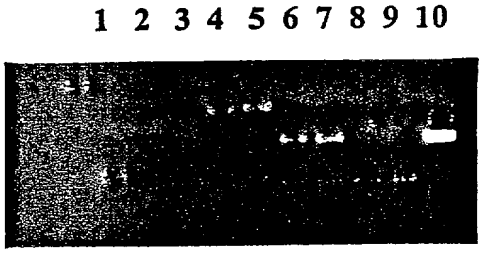
Unspliced genomic fragment
Spliced transcript

Figure 15



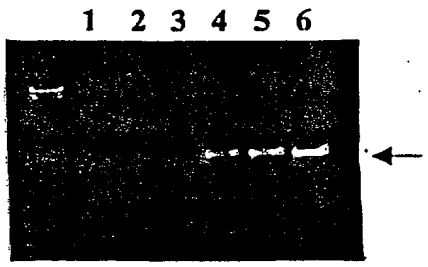
1. Low Mass Ladder: 2.0, 1.2, 0.8,0.4, 0.2 and 0.1kb
2. Normal Bovine spleen cDNA negative control
3. ΔΔHAC 5868A spleen cDNA
4. empty
5. Hi Lo :10.0,6.0,4.0,3.0,2.0,1.5,1.4,1.0,0.7,0.5,0.4,0.3, 0.2,0.1kb
6. Tc Mouse HAC spleen cDNA positive control
7. GAPDH product from 5868A spleen cDNA
8. GAPDH product from normal bovine spleen cDNA

Figure 16



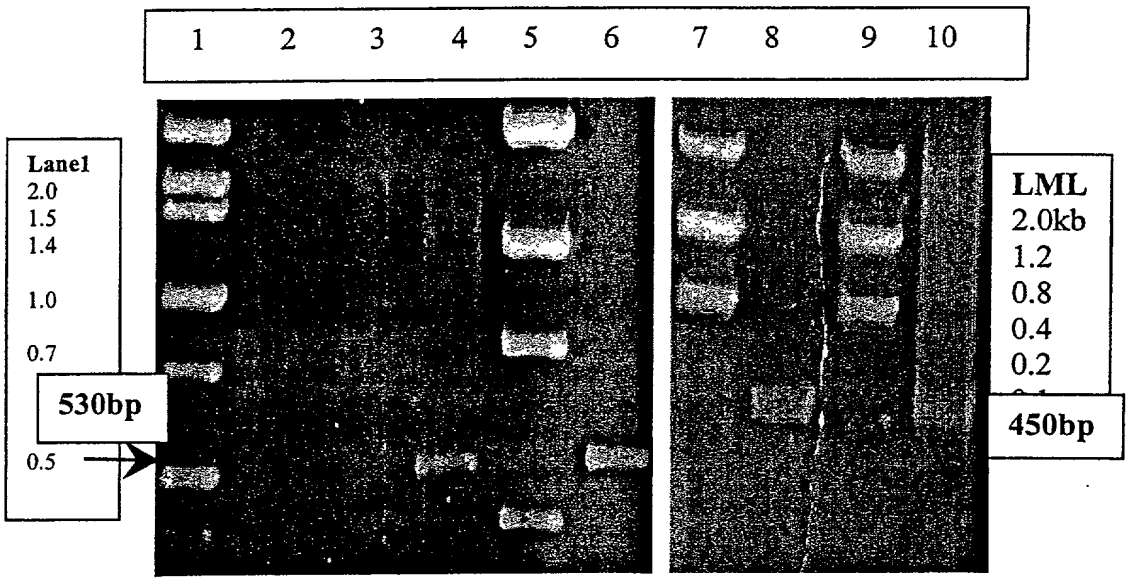
1. Bovine spleen (negative control)
2. Fetus 5442A brain
3. Fetus 5442B brain
4. Fetus 5442A liver
5. Fetus 5442B liver
6. Fetus 5442A spleen
7. Fetus 5442A spleen
8. Fetus 5442B spleen
9. Fetus 5442B spleen
10. ΔHAC-chimeric mouse spleen (positive control)

Figure 17



1. Bovine spleen (negative control)
2. Fetus 5442A brain
3. Fetus 5442A liver
4. Fetus 5442A spleen
5. Fetus 5442A spleen
6. ΔHAC-chimeric mouse spleen (positive control)

Figure 18



1. Hi-Lo MW:2.0,1.5,1.4,1.0,0.7,0.5 kb
2. ΔΔHAC 5868A fetal brain cDNA
3. ΔΔHAC 5868A fetal liver cDNA
4. ΔΔHAC 5868A fetal spleen cDNA
5. Low Mass Ladder
6. Tc Mouse HAC spleen cDNA positive control (**530bp**)
7. Low Mass Ladder
8. GAPDH ΔΔHAC 5868A brain cDNA
9. Low Mass Ladder
10. GAPDH ΔΔHAC 5868A liver cDNA

Figure 19

SEQ ID NOs: 56 and 57

```
5' ACC CTC CTC ACT CAC TGT GCA GGG TCC TGG GCC CAG TCT GTG CTG ACT CAG CCA
   T   L   L   T   H   C   A   G   S   W   A   Q   S   V   L   T   Q   P

   CCC TCA GCG TCT GGG ACC CCC GGG CAG AGG GTC ACC ATC TCT TGT TCT GGA AGC
    P   S   A   S   G   T   P   G   Q   R   V   T   I   S   C   S   G   S

   AGC TCC AAC ATC GGA AGT AAT TAT GTA TAC TGG TAC CAG CAG CTC CCA GGA ACG
    S   S   N   I   G   S   N   Y   V   Y   W   Y   Q   Q   L   P   G   T

   GCC CCC AAA CTC CTC ATC TAT AGG AAT AAT CAG CGG CCC TCA GGG GTC CCT GAC   V1-17
    A   P   K   L   L   I   Y   R   N   N   Q   R   P   S   G   V   P   D

   CGA TTC TCT GGC TCC AAG TCT GGC ACC TCA GCC TCC CTG GCC ATC AGT GGG CTC
    R   F   S   G   S   K   S   G   T   S   A   S   L   A   I   S   G   L

   CGG TCC GAG GAT GAG GCT GAT TAT TAC TGT GCA GCA TGG GAT GAC AGC CTG AGT
    R   S   E   D   E   A   D   Y   Y   C   A   A   W   D   D   S   L   S

   GGT CTT TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA GGT CAG CCC AAG GCT GCC
    G   L   F   G   G   G   T   K   L   T   V   L   G   Q   P   K   A   A
                            JL3

   CCC TCG GTC ACT CTG TTC CCA CCC TCC TCT GAG GAG CTT CAA GCC AAC AAG GCC
    P   S   V   T   L   F   P   P   S   S   E   E   L   Q   A   N   K   A
                                        Cλ

   ACA CTG GTG 3'
    T   L   V
```

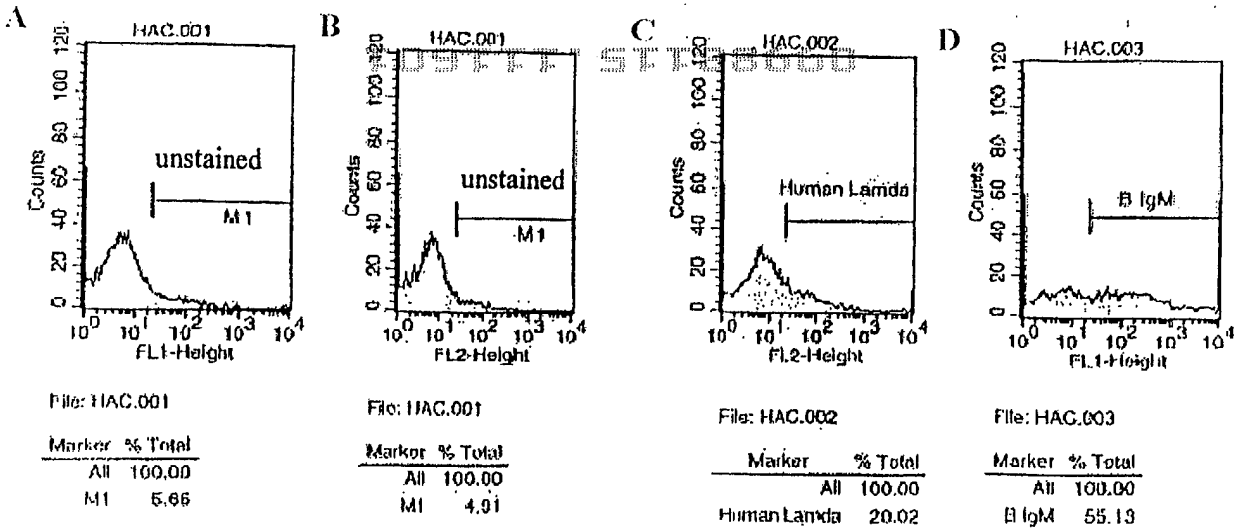
Figure 20

SEQ ID NOs: 58 and 59

```
5'  AGT TGG ACC CCT CTC TGG CTC ACT CTC TTC ACT CTT TGC ATA GGT TCT
      S   W   T   P   L   W   L   T   L   F   T   L   C   I   G   S

    GTG GTT TCT TCT GAG CTG ACT CAG GAC CCT GCT GTG TCT GTG GCC TTG GGA CAG
      V   V   S   S   E   L   T   Q   D   P   A   V   S   V   A   L   G   Q

    ACA GTC AGG ATC ACA TGC CAA GGA GAC AGC CTC AGA AGC TAT TAT GCA AGC TGG
      T   V   R   I   T   C   Q   G   D   S   L   R   S   Y   Y   A   S   W

    TAC CAG CAG AAG CCA GGA CAG GCC CCT GTA CTT GTC ATC TAT GGT AAA AAC AAC   V2-13
      Y   Q   Q   K   P   G   Q   A   P   V   L   V   I   Y   G   K   N   N

    CGG CCC TCA GGG ATC CCA GAC CGA TTC TCT GGC TCC AGC TCA GGA AAC ACA GCT
      R   P   S   G   I   P   D   R   F   S   G   S   S   S   G   N   T   A

    TCC TTG ACC ATC ACT GGG GCT CAG GCG GAA GAT GAG GCT GAC TAT TAC TGT AAC
      S   L   T   I   T   G   A   Q   A   E   D   E   A   D   Y   Y   C   N

    TCC CGG GAC AGC AGT GGT AAC CAT CTG GTA TTC GGC GGA GGG ACC AAG CTG ACC
      S   R   D   S   S   G   N   H   L   V   F   G   G   G   T   K   L   T   JL2

    GTC CTA GGT CAG CCC AAG GCT GCC CCC TCG GTC ACT CTG TTC CCA CCC TCC TCT
      V   L   G   Q   P   K   A   A   P   S   V   T   L   F   P   P   S   S

    GAG GAG CTT CAA GCC AAC AAG GCC ACA CTG GTG 3'
      E   E   L   Q   A   N   K   A   T   L   V

                    Cλ
```

Figure 21

Fetus #5442A



HAC-B

Fetus #5442B

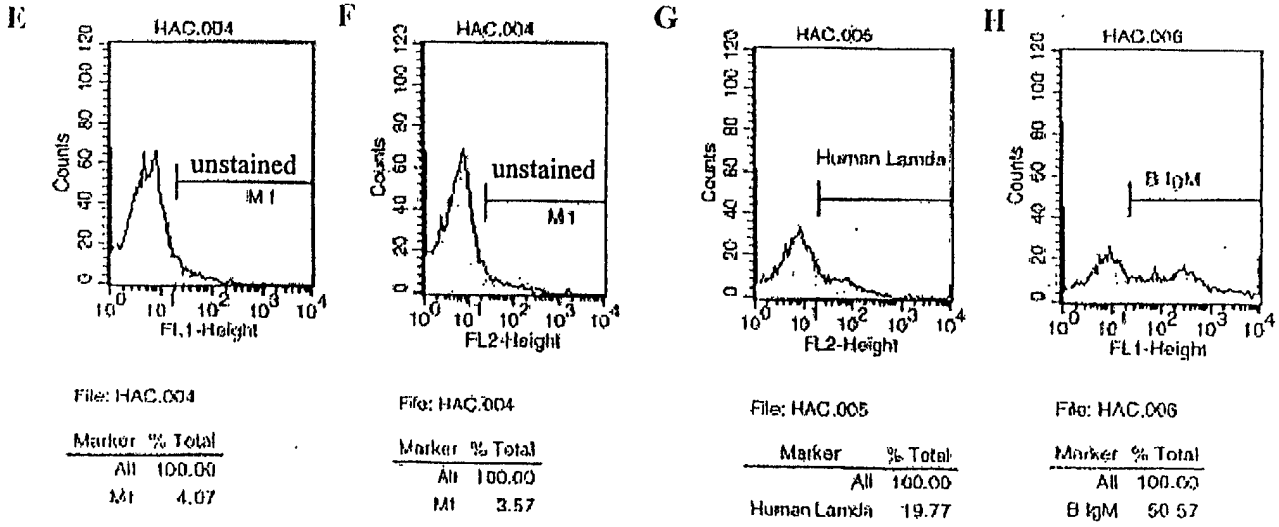


Figure 22A - 22H

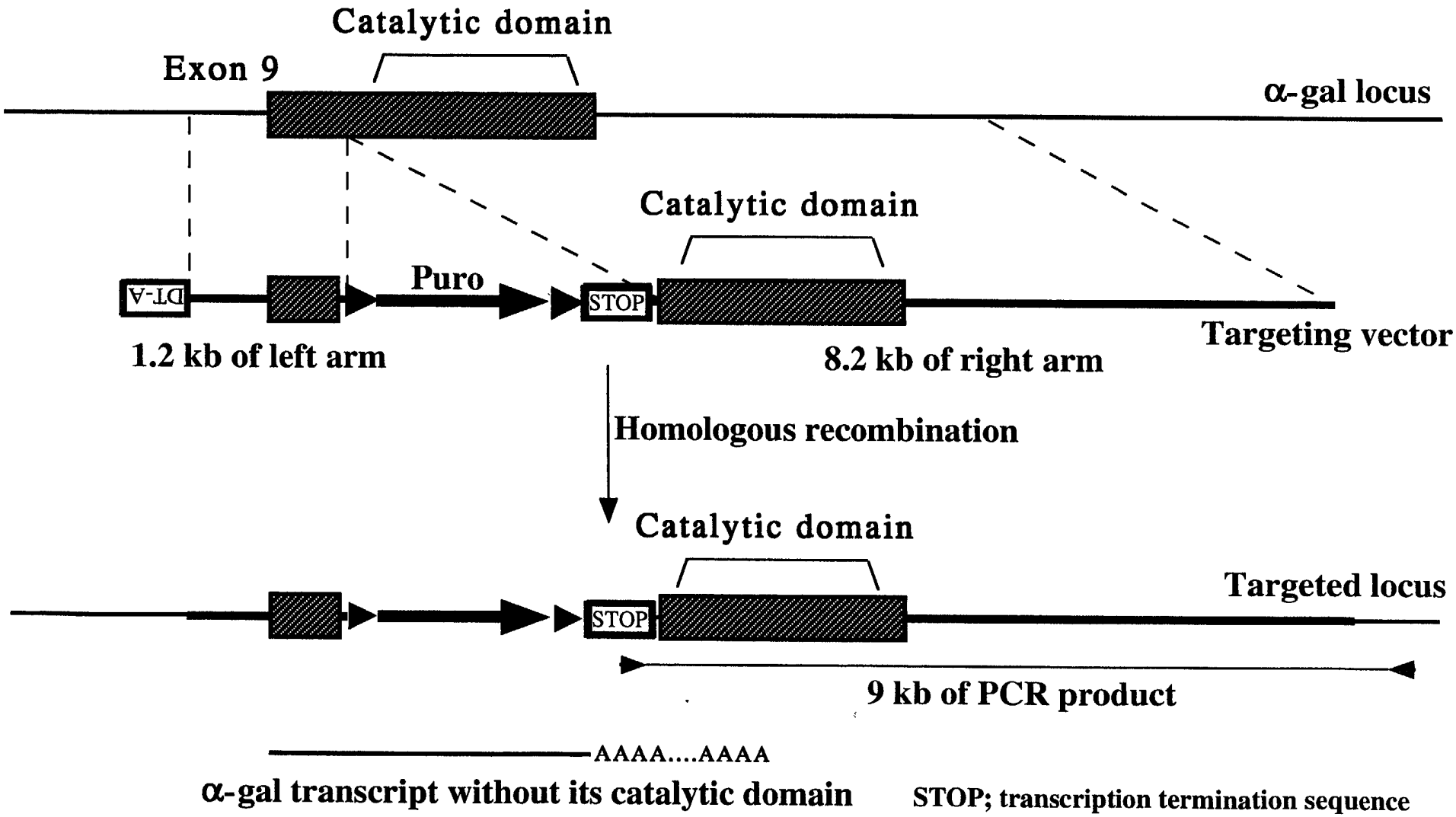
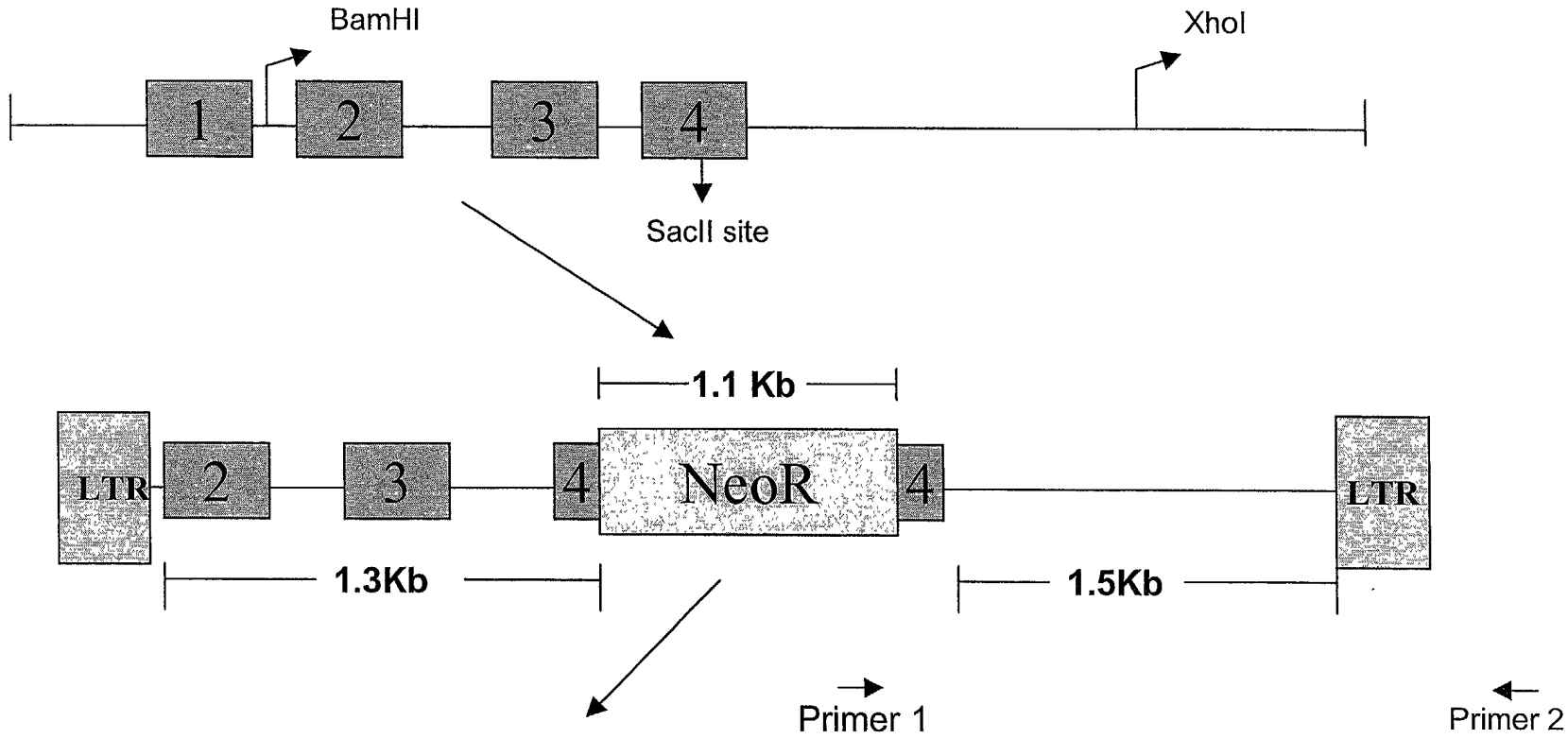

Catalytic domain
Exon 9
α-gal locus
Catalytic domain
DT-A
Puro
STOP
1.2 kb of left arm
8.2 kb of right arm
Targeting vector
Homologous recombination
Catalytic domain
Targeted locus
STOP
9 kb of PCR product
AAAA....AAAA
α-gal transcript without its catalytic domain
STOP; transcription termination sequence
DT-A; diphtheria toxin gene
Puro; puromycin-resistant gene

Figure 23



PACKAGE AAV PARTICLES

Figure 24

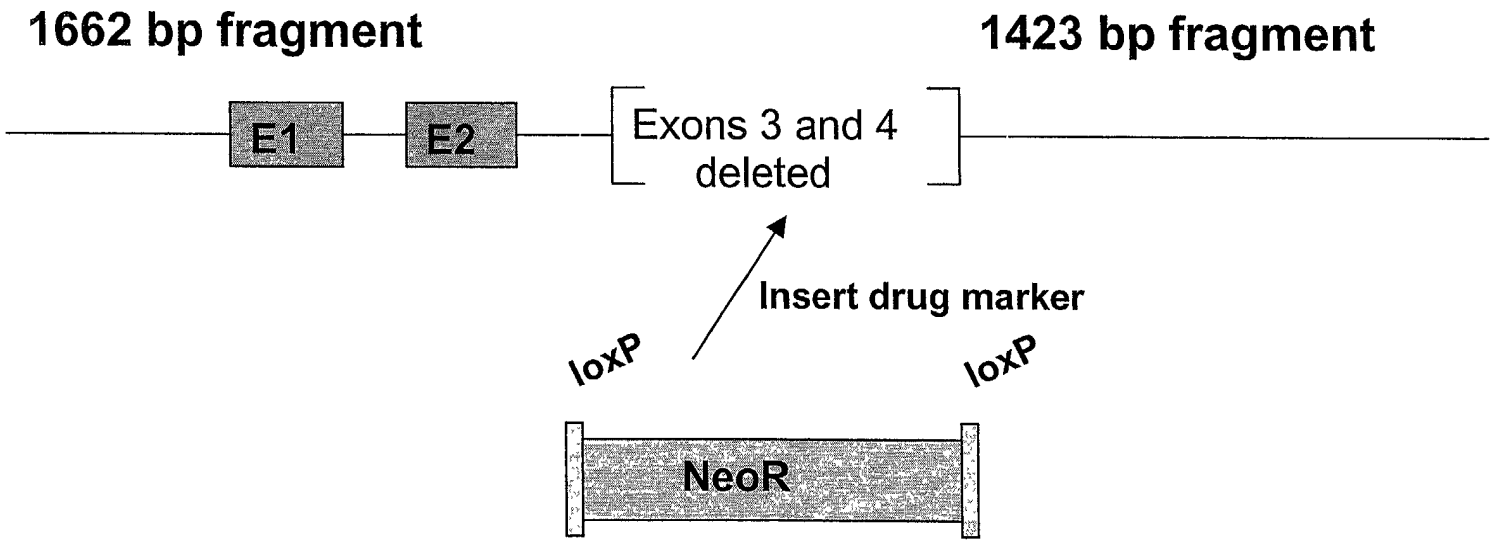

1662 bp fragment
1423 bp fragment
E1
E2
Exons 3 and 4
deleted
Insert drug marker
loxP
loxP
NeoR

Figure 25

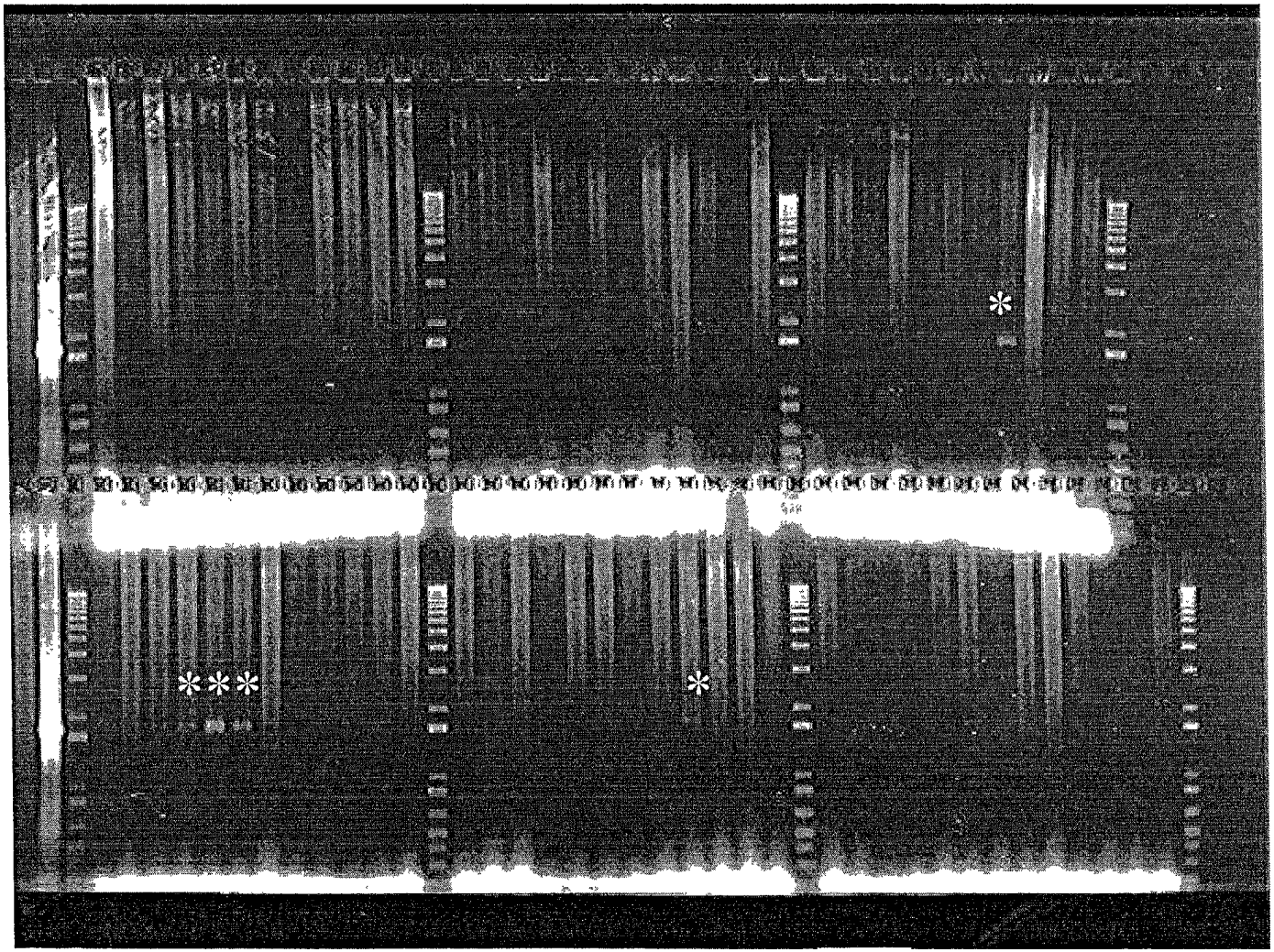


Figure 26

NT, ET and pregnancies :Delta HAC regenerated fibroblasts

Cell line ID	Total NTs in culture	No of Blast (%)	No of Blast Tranferred	No Recips	Pregnancy status 40 d	60 d	90 d	120 d	150 d	180 d	210 d
D5968	174	34 (28)	27	17	3	3	3	3	3	3	3
D6045	215	10 (7)	8	4	1	1	1	1	1	1	
D6045	122	20 (23)	12	9	1	0	0	0	0	0	
D6032	161	18 (16)	14	7	3	3	3	2	2	2	
D6032	188	15 (11)	11	11	3	0	0	0	0	0	
D6032	198	20 (14)	16	10	1	1	1	1			
D6032	200	17 (12)	12	8	2	2	2	2			
D6032	180	11 (9)	10	5	3	1	1	0			
D6032	135	22 (23)	22	11	2	1	1	1			
D5968	140	35 (36)	25	13	2	2	1				
D5968	180	30 (24)	26	13	2	2	1				
D6045	170	46 (39)	32	16	4						
D6045	80	7 (13)	1	1	0						
D6045 SLOT	108	9 (12)	3	2	1						
D6045	76	8 (15)	2	1	0						
D6045 SLOT	128	12 (13)	7	5	0						
D6045	47	6 (18)	5	3	2						
D6045 SLOT	112	3 (4)	3	2	2						
D6045	120	28 (33)	18	9							
D6045 SLOT	100	11 (16)	2	1							
D6045	78	15 (27)	16	8							
D6045 SLOT	91	0	2	1							
D6045	98	16 (23)	10	5							
D6045 SLOT	104	16 (22)	10	5							
D5968	128	24 (27)	8	4							
D5968 SLOT	65	10 (22)	8	4							
D5968	120	28 (33)	14	7							
D5968 SLOT	95	13 (19)	6	3							
D5968	98	17 (25)	20	10							
D5968 SLOT	93	14 (22)	12	6							
D	13	1 (11)	1	3							
SLOT	63	8 (18)	8	3							
D	108	4 (5)	4	3							
SLOT	100	1 (1)	1	3							
D	90	10 (16)	10	6							
SLOT	110	13 (17)	13	6							
D	90	10 (16)	10	1							
SLOT	83	5 (9)	5	1							
D	105	20 (27)	20	9							
SLOT	78	7 (13)	7	2							
D	88	7 (11)	7	4							
SLOT	93	9 (14)	9	4							
D	85	20 (33)	20	10							
SLOT	77	4 (7)	4	2							
	4987	**515 (19)**	**481**	**258**							

Summary

Preg Status	*No of Pregnancies*
> 40 d	9
> 90 d	2
> 120 d	4
> 180 d	3
> 210 d	3
Total	**21**

Figure 27